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**Plasma Syndecan-1 in Hemodialysis Patients Associates with Survival and Lower  
Markers of Volume Status**

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22

23 **Author contributions**

24 J. Koch, S. Assa, C.F.M. Franssen and J. van den Born conceived and designed the study;  
25 S. Assa and C.F.M. Franssen executed the clinical part of the study; J. Koch, N.M.A. Idzerda  
26 and W. Dam performed the laboratory measurements; J. Koch, N.M.A. Idzerda, C.F.M.  
27 Franssen and J. van den Born analyzed and interpreted the data and drafted the manuscript.  
28 All authors approved the final version of the manuscript.

**Abstract**

Syndecan-1, a transmembrane heparan sulfate proteoglycan, associates with renal and cardiovascular functioning. We earlier reported syndecan-1 to be involved in renal tubular regeneration. We now examined plasma values of syndecan-1 in a hemodialysis cohort and its association with volume, inflammatory and endothelial markers in addition to outcome.

Eighty-four prevalent hemodialysis patients were evaluated for their plasma syndecan-1 levels by ELISA before the start of HD, as well as 60, 180 and 240 minutes after starting dialysis. Patients were divided into sex-stratified tertiles based on predialysis plasma syndecan-1 levels. We studied the association between plasma levels of syndecan-1 and volume, inflammation and endothelial markers and its association with cardiovascular events and all-cause mortality using Kaplan-Meier curves and cox regression analyses with adjustments for gender, age, diabetes and dialysis vintage.

Predialysis syndecan-1 levels were two-fold higher in males compared to females ( $P=0.0003$ ). Patients in the highest predialysis plasma syndecan-1 tertile had a significantly higher ultrafiltration rate ( $P=0.034$ ) and lower plasma values of BNP ( $P=0.019$ ), pro-ANP ( $P=0.024$ ) and endothelin ( $P<0.0001$ ) compared with the two lower predialysis syndecan-1 tertiles. No significant associations with inflammatory markers were found. Cox regression analysis showed that patients in the highest syndecan-1 tertile had significantly less CV events and better survival compared with the lowest syndecan-1 tertile ( $P=0.02$  and  $P=0.005$ , respectively).

In hemodialysis patients, higher plasma syndecan-1 levels were associated with lower concentrations of BNP, pro-ANP and endothelin, and with better patient survival. This may suggest that control of volume status in hemodialysis patients allows an adaptive tissue regenerative response as reflected by higher plasma syndecan-1 levels.

**Key words:** hemodialysis; ultrafiltration; inflammation; syndecan-1.

## **Introduction**

Hemodialysis (HD) patients have a strongly increased cardiovascular (CV) mortality rate compared to age- and sex-matched healthy individuals. Various factors contribute to this increased CV mortality, among others uremia, inflammation (1), endothelial dysfunction, hypertension (2), and volume overload (3), all contributing to accelerated atherosclerosis (4). Moreover, HD is an inflammatory trigger and induces oxidative stress (5,6) and endothelial dysfunction (6,7). We hypothesized that in HD patients an adaptive tissue repair response is induced secondary to tissue injury caused by the aforementioned factors related to renal failure including volume overload and by HD-induced inflammation, oxidative stress, and endothelial dysfunction. Syndecan-1 is suggested as a tissue repair response marker. Upon injury, syndecan-1 is upregulated and involved in tissue repair responses in different organs such as the skin (8) or the kidneys (9,10). As a result of cellular shedding, an increase of plasma syndecan-1 levels can be acutely induced by a number of different stimuli such as major surgery, inflammatory insults, ischemia-reperfusion, shock, and also during HD (11,12), leading to a lower responsiveness status of the cells of origin. In vitro, syndecan-1 shedding can be induced by pro-inflammatory cytokines (13) and a number of growth factors (14). Syndecan-1 is one of the four transmembrane heparan sulfate proteoglycans (HSPGs) of the syndecan family. All family members contain a transmembrane core protein to which glycosaminoglycan side chains are extracellularly attached (15). Syndecan-1 is expressed in many cell types during development but is down-regulated in most cells after birth (16). In adults, syndecan-1 is mainly expressed by hepatocytes (17), epithelial cells (18) and plasma cells (19). Elevated plasma syndecan-1 is usually interpreted as endothelial glycocalyx loss, and/or shedding from the (renal) epithelium and/or the liver (9,20,21). Shedding is executed by matrix metalloproteinases (MMPs) and by disintegrin and metalloproteinases (ADAMs), such as MMP9 and ADAM17 (22). Previous research showed syndecan-1 to be anti-apoptotic, anti-inflammatory and anti-fibrotic (9,10).

In contrast to acutely-induced syndecan-1 shedding, in chronic disease, tissue syndecan-1 expression reflects an adaptive healing response to the underlying insult, such

as renal transplantation (10), heart failure (23), and HD treatment (20). As such, plasma syndecan-1 levels are believed to reflect tissue expression levels of syndecan-1 as the result of normal turnover (24). However, after initial increase, when damage progressively increases, tissue syndecan-1 expression diminishes again and cell death and fibrosis take over. Various renal models showed reduced repair and more inflammation and fibrosis in syndecan-1 knock-out (KO) mice compared to wild type (WT) animals (10). This is due to its crucial co-receptor function for regenerative growth factors such as vascular endothelial growth factor (VEGF) (25), insulin-like growth factor (IGF) (26), and its association with various integrins (27). By that, syndecan-1 is orchestrating proliferation (25), migration (28) and adhesion (29). Thus, depending on the stage and severity of the tissue damage, a positive association (initial disease) or a negative association (progressed disease) is found between injury and plasma (or tissue) syndecan-1 (9).

Since adaptive tissue repair responses are relevant in the context of HD, the primary study question was whether pre- and intradialytic plasma syndecan-1 levels in stable HD patients associate with volume, inflammatory and endothelial markers and with outcome.

99

100 **Patients and Methods:**

101 Patients and Study Design

102 This study is a post-hoc analysis of the study by Assa et al. (30). In short, HD patients from  
103 the Dialysis Center Groningen and University Medical Center Groningen were eligible for this  
104 study if they were treated with HD for more than 3 months and were on a thrice-weekly HD  
105 schedule. The patients had end-stage chronic kidney disease due to hypertension (n=17),  
106 diabetes (n=12), adult dominant polycystic kidney disease (n=8 ), focal segmental  
107 glomerulosclerosis (n=7), IgA nephropathy (n=4), chronic pyelonephritis (n=2),  
108 glomerulonephritis (n=10), chronic obstructive nephropathy (n=4), other diagnoses (n=8) and  
109 unknown etiology (n=12). The median dialysis vintage was 2.1 years (interquartile range: 0.8  
110 to 4.4 years). Patients with severe heart failure (NYHA stage IV) and patients that did not  
111 have an adequate window for echocardiography imaging were excluded. The original cohort  
112 consisted of 109 patients on conventional HD. Twenty-five subjects were excluded for the  
113 present study due to lack of sufficient plasma for syndecan-1 measurements. All patients  
114 gave written informed consent for participation in this study. The study was performed  
115 according to the principles of the Declaration of Helsinki and was approved by the local  
116 Medical Ethical Committee. The study took place from March 2009 until March 2010. The  
117 follow-up for survival and CV events was 4 years.

118

119 Study Protocol

120 Patients were studied at the first dialysis session of the week. Dialysis duration was 4 hours.  
121 The assessment of patients' characteristics took place at study entry. The definition of  
122 diabetes was a fasting blood glucose level of >7 mmol/L or the use of antidiabetic drugs.  
123 Hypertension was defined as predialysis systolic blood pressure above 140 mm Hg and/or  
124 diastolic blood pressure of higher than 90 mm Hg and/or the use of antihypertensive drugs.  
125 We defined cardiovascular history as a history of ischemic heart disease, congestive heart  
126 failure, coronary artery bypass grafting, percutaneous coronary intervention, stroke, or

peripheral vascular disease. These data were obtained from patients' medical records. Heart rate and blood pressure were measured before and after HD. UF rate was expressed as milliliters per hour per kilogram body weight by dividing UF volume by dialysis session length and postdialysis target weight (31). Nutritional status was assessed with the 7-point subjective global assessment (SGA). Patients were defined as malnourished if SGA was  $\leq 5$ .

### Laboratory Procedures

Arterial blood was taken from the arterial line before the start of HD, and during HD which was at 60, 180 and 240 minutes after the start of HD. Hematocrit, leukocytes, neutrophils, and albumin were measured immediately by routine diagnostics. To determine cytokine levels, the blood was centrifuged 30 minutes at 3.500 rpm for 15 minutes, after which the plasma fraction was taken and stored at  $-80^{\circ}\text{C}$ . Samples were thawed and recentrifuged before measurements. Syndecan-1 concentrations were measured in EDTA plasma samples using sCD138 sandwich ELISA kits (Diacclone, Besancon, France) according to the manufacturer's instructions. High-sensitivity C-reactive protein (hs-CRP) was measured by CRP monoassay (Siemens Healthcare Diagnostics, Newark, DE, USA). Quantikine sandwich enzyme immunoassay technique (R&D Systems Inc., Minneapolis, MN, USA) was used for measuring Pentraxin 3 (PTX3), interleukin 6 (IL-6), interleukin-10 (IL-10), soluble intercellular adhesion molecule 1 (ICAM-1). TNF- $\alpha$  was measured by Quantikine HS Human immunoassay (R&D system, Minneapolis, MN, USA). Von Willebrand factor was measured by enzyme-linked immunosorbent assay (Dakopatts, Glostrup, Denmark). Endothelin measurement took place by competing with surface-bound recombinant endothelin (RayBiotech Inc., Norcross, GA, USA) for binding to a specific antibody (RayBiotech Inc.). By using substrate conversion of a horseradish peroxidase-labeled secondary antibody we measured the amount of captured antibody. Proendothelin measurement was done by novel sandwich fluoroimmunoassay (BRAHMS, Hennigsdorf/ Berlin, Germany) using the automated system B-R-A-H-M-S KRYPTOR. Plasma angiopoietin-1 (Ang1) and Ang2 levels were measured via enzyme-linked immunosorbent assay (ELISA) Duosets (R&D systems,



Minneapolis, USA). The concentration of all biomarkers that were measured during and after dialysis was corrected for the effect of hemoconcentration according to Schneditz et al. (32). All stored samples were analyzed at the same time to reduce interassay variability. Laboratory staff was not aware of patient data or outcome.

### Statistical Analyses

Analyses were performed with IBM SPSS software version 24.0 (IBM, Armonk, NY, USA), GraphPad Prism version 7.00; GraphPad Software (La Jolla, CA, USA) and R version 3.3.1 (The R Foundation for Statistical computing). Continuous variables with normal distributions are reported as mean  $\pm$  standard deviation (SD), skewed variables as median and interquartile range and categorical data as number and percentage. Normality was tested with the Shapiro-Wilk test. A (non-parametric) Levene's test was used to verify the equality of variances in the data. Correlations between nonparametric variables were calculated using the Spearman rank correlation coefficient. Comparisons were made with a Wilcoxon Signed Rank Test, Mann-Whitney U test, Kruskal-Wallis test or one-way analysis of variance (ANOVA) when appropriate. A Kaplan Meier analysis was used to explore the occurrence of CV events and mortality across tertiles of baseline syndecan-1 levels. The hazard ratios for cardiovascular events and mortality across these tertiles were estimated by a Cox proportional hazard regression model, with adjustment for gender, age, diabetes and dialysis vintage. Here, log-transformation of syndecan-1 was performed to ensure a linear relationship between the endpoint and predictor variables. Two-sided  $P < 0.05$  was considered statistically significant.

## Results

### Patients

The characteristics of the 84 patients in this study are shown in Table 1. One third of the participants were female. The mean ( $\pm$ SD) age of all patients was  $63\pm 16$  years. Predialysis syndecan-1 levels showed a skewed distribution (Figure 1a) and were not associated with the underlying disease or the use of medications (data not shown). However syndecan-1 levels were found to be two-fold higher in male compared to female patients ( $P=0.0003$ ) (Figure 1b). Therefore, patients were categorized into sex-stratified tertiles according to their plasma syndecan-1 levels, with tertile 1 having low, tertile 2 having intermediate and tertile 3 having high syndecan-1 values (Table 1).

### Associations with predialysis syndecan-1

Age, BMI, body weight, SGA and blood pressure did not differ significantly between syndecan-1 tertiles (Table 1). Patients in tertile 3 had significantly lower values of BNP ( $P=0.019$ ) and pro-ANP ( $P=0.024$ ) compared with tertile 1 and 2. Levels of endothelin were significantly lower ( $P<0.0001$ ) in tertile 3 compared with tertile 1 and 2. Spearman rank correlation analysis revealed an inverse correlation of predialysis plasma syndecan-1 with endothelin ( $R=-0.261$ ;  $P=0.016$ ) and BNP ( $R=-0.222$ ;  $P=0.042$ ). The inflammatory markers CRP, IL-6 and TNF- $\alpha$  were non-significantly lower in tertile 3 compared with tertile 1 and 2 (Table 1).

### Predialysis syndecan-1 and its association with survival and cardiovascular events

Kaplan-Meier analysis of the predialysis syndecan-1 tertiles demonstrated significantly better survival ( $P=0.003$ ) of the patients in tertile 3 compared with tertile 1 (Figure 2a). Patients in tertile 3 also had fewer CV events compared with tertile 1 ( $P=0.01$ ) (Figure 2b). Notably, the survival curves began to separate relatively early during follow-up. Cox regression analysis showed that also after adjustment for age, sex, diabetes and dialysis vintage patients in

tertile 3 (hazard ratio for mortality 0.3) had a significantly better survival compared with those in tertile 1 (hazard ratio for mortality 2.1) ( $P=0.005$ ). For the same adjustments, cox regression also showed significantly lower CV events in tertile 3 (hazard ratio for CV events 0.3) compared to tertile 1 (hazard ratio for CV events 1.3) ( $P=0.02$ ).

#### Associations of syndecan-1 with volume and inflammatory markers during hemodialysis

Figure 3a depicts the intradialytic course of plasma syndecan-1 levels. In tertile 3, syndecan-1 levels rose by 18%, although not significant ( $p=0.7786$ ). In contrast, levels rose significantly by 6% and 14% in tertile 1 ( $p<0.0001$ ) and tertile 2 ( $p=0.0147$ ), respectively. Values remained significantly different from each other at 240 minutes after start of dialysis. During HD, hematocrit, endothelin, pro-ANP, and CRP values increased whereas BNP and IL-6 levels decreased (not significant). Patients in tertile 3 had higher hematocrit (not significant) (Figure 3b) throughout HD as compared with patients in tertile 1. In contrast, patients in tertile 3 had lower values of endothelin (not significant) (Figure 3d), BNP ( $P=0.00079$ ) (Figure 3e), pro-ANP ( $P=0.0269$ ) (Figure 3f), IL-6 (not significant) (Figure 3g), and CRP (not significant) (Figure 3h) in comparison with the patients in tertile 1 and/or 2. UF rate (Figure 3g) was significantly higher in tertile 3 compared to tertile 1 ( $P=0.034$ ) (Figure 3c).

## **Discussion**

The major findings of this study are that higher plasma syndecan-1 levels associate with lower plasma levels of BNP, pro-ANP and endothelin, and with higher ultrafiltration rates during HD. Patients in the highest plasma syndecan-1 tertile also had a significantly lower all-cause mortality and a lower incidence of CV events.

From previous research it is known that dialysis patients have a high incidence of CV events and related mortality (33). This has been linked to uremia, chronic inflammation (1), oxidative stress (5), volume overload (3) and endothelial dysfunction (7). The repair response of tissue damage depends on intrinsic regenerative capacity and stem cells (34), the latter being reduced in patients with renal failure including HD patients (35). Increased markers of tissue repair have been reported in several renal studies such as VEGF (9), heparin-binding EGF (36), and syndecan-1 (9). Just like endothelial markers such as CD31 (37), increased plasma levels of syndecan-1 have been documented earlier in HD studies where it has been interpreted as an indicator for endothelial glycocalyx damage (20).

According to our previous observations (9), the syndecan-1 response is bi-phasic upon increasing injury. With initial injury, a tissue repair process is triggered where syndecan-1 plays a role which is reflected by higher tissue as well as plasma syndecan-1 values. However, upon progression and chronic development of the underlying damage, cellular syndecan-1 expression is lost again resulting in an inverse association of syndecan-1 and disease parameters (9). In many situations, where tissue injury is less severe, a positive association of tissue degradation with syndecan-1 has been reported (10,20). Our research showed that the patients with the highest plasma syndecan-1 values (tertile 3) had better survival and fewer CV events. We therefore consider them to have a better clinical and tissue condition. We could not confirm this by the SGA and inflammatory markers which, however, showed a trend that support our findings. The CRP and IL-6 tended to be lower in tertile 3 compared with tertile 1, both before and during HD. Furthermore, we found that the patients in tertile 3 had lower endothelin values suggesting less endothelial dysfunction (38). Also,

BNP and pro-ANP values were lower in this patient group. These markers are released from the ventricles and right atrium following wall stress due to hypervolemia (39). Moreover, patients in tertile 3 also had a higher ultrafiltration rate. These data suggest that the reduced volume status in the highest syndecan-1 tertile is the consequence of better volume control during dialysis. Thus, our findings might suggest that lower extracellular volume in dialysis patients favors the adaptive tissue regenerative response as reflected by higher plasma syndecan-1 levels. This theory would be in accordance with the findings of Gunal and colleagues (40). Collectively, these data show that the patients with high plasma syndecan-1 values represent patients with lower extracellular volume and less inflammation and endothelial dysfunction resulting in improved patient survival.

At this point, the origin of plasma syndecan-1 in HD patients and the mechanism of its increased expression and shedding can only be speculated but is often interpreted as endothelial glycocalyx loss, and/or shedding from the (renal) epithelium and/or the liver (9,20,21). Previous publications indicate that syndecan-1 transcription is regulated by the proinflammatory transcription factor NF- $\kappa$ B and fibroblast growth factor-inducible response element that is located on the upper syndecan-1 promoter. Besides, the induction of syndecan-1 mRNA expression by transforming growth factor as well as EGF has been shown in vitro. (41) These data indicate syndecan-1 induction under conditions of inflammation and repair, which is relevant in the context of HD. Adepu et al. (9) reported enhanced ADAM17 expression in a renal transplantation model in the rat which is a major syndecan-1 sheddase, but other MMPs could play a role as well (22). A possible trigger of syndecan-1 shedding is not yet clear. However, systemic inflammation induced by allograft transplantation showed shedding by proteases such as ADAM17 and an increase of plasma syndecan-1 (13,42). In our cohort we demonstrated an increase of inflammation and syndecan-1 during HD. In vitro studies have shown that inflammatory cytokines, particularly IL-1, IL-6, and TNF- $\alpha$ , as well as reactive oxygen species are involved in the degradation of hyaluronan, a major constituent of the glycocalyx (43).

Nevertheless, syndecan-1 has been shown to be expressed by hepatocytes (17), epithelial cells (18) and plasma cells (19), but not yet on endothelial cells in vivo (9). Moreover, we could not find significant associations of plasma syndecan-1 with the endothelial markers ICAM-1, von Willebrand factor, Ang1 and 2. We therefore suggest that plasma syndecan-1 arises from non-endothelial origin, most likely the liver, epithelial tissues and plasma cells (9,21).

In this study, we found a significant difference in plasma levels of syndecan-1 between men and women. This could be explained by different dietary habits (44) and/or differences in sex-hormones (45) as has been reported before in chronic kidney disease patients.

There are some important limitations to the present study. First, the number of patients was relatively small. Second, no healthy controls were included to extend the reproduction of earlier studies. Another limitation is that we measured plasma syndecan-1 only once (at baseline). Future studies should investigate whether plasma syndecan-1 levels change over time and if such changes are related to the patients' clinical situation.

We conclude that in hemodialysis patients, higher plasma syndecan-1 levels were associated with lower concentrations of markers of volume status and endothelin, and with better patient survival. This may suggest that control of volume status in hemodialysis patients allows an adaptive tissue regenerative response as reflected by higher plasma syndecan-1 levels. This argues for strict extracellular volume control in HD patients. More research needs to be done to explore the origin and the (patho-) physiologic roles of syndecan-1 in HD patients. Lastly, we cannot exclude the possibility that the loss of tissue syndecan-1 expression, resulting in low plasma values, induces a pro-inflammatory condition associated with increased extracellular volume.

305

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309

310 **Disclosures**

311 None to declare.

312

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441  
442 **Figure 1: Distribution of predialysis plasma syndecan-1 in stable hemodialysis**  
443 **patients. (a)** Number of patients (frequency) and the skewed distribution of their predialysis  
444 plasma syndecan-1 levels (ng/ml). **(b)** Distribution of predialysis plasma syndecan-1 levels  
445 (ng/ml) for all patients and for men and women, respectively (median and interquartile range)  
446 showing men to have significantly higher plasma syndecan-1 levels compared to women.  
447 Mann-Whitney U test \*\*\*P=0.0003.

448

449

450 **Figure 2: Predialysis plasma syndecan-1 is associated with survival and lower**  
451 **cardiovascular events. (a)** Kaplan-Meier survival curve showing the association between  
452 predialysis plasma syndecan-1 (indicated in tertiles) and overall survival. \*\*Log-rank test  
453  $P=0.003$  **(b)** Kaplan-Meier curve depicting the association between predialysis plasma  
454 syndecan-1 and CV events. \*Log-rank test  $P=0.01$ .

455

456

457 **Figure 3: Volume, endothelial and inflammatory parameters shown in plasma**  
458 **syndecan-1 tertiles during hemodialysis session.** Mann-Whitney U test for comparing  
459 different groups. Wilcoxon signed rank test for comparing within the same group. (a)  
460 Syndecan-1 increases over time with tertile 1 (\*\*\*\*P<0.0001) and 2 (\*P=0.0147) being  
461 statistically significant. At 240 minutes, all tertiles are statistically different from each other,  
462 with tertile 3 showing the highest values. Tertiles 1 and 2 \*\*P=0.0017. Tertiles 1 and 3  
463 \*\*\*\*P<0.0001. Tertiles 2 and 3 \*\*\*P=0.0003. Hematocrit (b) increase over time where tertile 3  
464 remains to have the highest values. Endothelin (d), pro-ANP (f), and CRP (h) increase over  
465 time with tertile 3 having the lowest values. Only pro-ANP is statistically significant (tertile 1  
466 and 3 \*P=0.0269). BNP (e) and IL-6 (g) decrease over time. Tertile 3 has lower values than  
467 tertile 2 with BNP being significant (\*\*P=0.0079). UFR (c) shows the highest values in tertile  
468 3. Tertile 1 and 3 being statistically significant (\*P=0.034).

1 Table 1. Patient characteristics according to sex-stratified tertiles of plasma syndecan-1.

	All	Tertile 1	Tertile 2	Tertile 3	p-value
<b>N</b>	84	27	28	29	
<b>Predialysis plasma syndecan-1 (ng/mL)</b>	48 (26 – 90)	21 (14 – 33)	51 (32 – 59)	133 (88 – 256)	<0.0001
Men (%)	65	67	64	66	
Plasma syndecan-1 in men (ng/mL)	57 (37 – 129)	28 (16 – 37)	56 (51 – 69)	157 (123 – 256)	<0.0001
Women (%)	35	33	36	34	
Plasma syndecan-1 in women (ng/mL)	30 (20 – 41)	17 (11 – 20)	29 (25 – 33)	50 (39 – 256)	<0.0001
<b>Patient characteristics</b>					
Age (years)	63 ± 16	66 ± 16	61 ± 14	61 ± 16	0.442
BMI (kg/m <sup>2</sup> )	25 (23 – 28)	24 (23 – 28)	26 (24 – 28)	24 (23 – 30)	0.462
SGA	6 (6 – 7)	6 (6 – 6)	6 (6 – 7)	6 (5 – 7)	0.922
Dialysis vintage (years)	2.1 (0.8 – 4.4)	3.9 (1 – 4.5)	2.1 (0.8 – 4.0)	2.2 (0.7 – 4.0)	0.463
Residual diuresis (ml/day)	0 (0 – 590)	0 (0 – 300)	0 (0 – 650)	0 (0 – 585)	0.792
Weight after HD (kg)	77 (68 – 88)	72 (64 – 81)	75 (67 – 89)	78 (73 – 88)	0.432
Intradialytic weight loss (kg)	1.95 (1.30 – 2.60)	1.80 (1.35 – 2.30)	2.05 (1.35 – 2.78)	2.00 (1.30 – 2.70)	0.468
Intradialytic weight loss/body weight	0.026 (0.018–0.034)	0.025 (0.018–0.030)	0.027 (0.019–0.037)	0.026 (0.016–0.034)	0.626

Predialysis systolic pressure (mmHg)	138 (124 – 155)	137 (124 – 163)	145 (125– 165)	141 (123 – 154)	0.600
Predialysis Diastolic pressure (mmHg)	79 (69 – 89)	78 (70 – 89)	80 (68 – 98)	78 (72 – 88)	0.831
Heart Rate (bpm)	73 ± 14	74 ± 14	73 ± 18	71 ± 13	0.500
<b>Inflammatory markers</b>					
CRP (mg/dl)	6.7 (2.5 – 11.8)	6.7 (2.1 – 18.4)	5 (2.6 – 8.8)	4.3 (1.2 – 8.5)	0.247
IL-6 (pg/ml)	5.8 (3.9 – 8.6)	7.7 (4.0 – 14.2)	5.4 (3.0 – 8.5)	5.4 (3.4 – 7.2)	0.113
IL-10 (pg/ml)	0.4 (0.3 – 0.6)	0.4 (0.3 – 0.8)	0.4 (0.3 – 0.5)	0.4 (0.3 – 0.6)	0.749
PTX3 (ng/ml)	2.57 (1.6 – 4.0)	2.6 (1.6 – 4.5)	2.5 (1.6 – 4.3)	2.4 (1.6 – 3.1)	0.729
TNF- $\alpha$ (pg/ml)	3.8 ± 1.9	4.4 ± 3.1	3.6 ± 1.2	3.5 ± 0.9	0.155
<b>Endothelial markers</b>					
Endothelin (pg/ml)	39 (24 – 66)	44 (29 – 72)	43 (25 – 68)	28 (15 – 47)	<0.0001
Pro-endothelin (pg/ml)	280 ± 69	285 ± 71	272 ± 55	284 ± 74	0.012
ICAM-1 (ng/ml)	141 (125 – 158)	140 (125 – 147)	149 (128 – 174)	138 (113 – 158)	0.320
Von Willebrand factor (%)	117 (93 – 148)	126 (102 – 146)	118 (90 – 150)	111 (93 – 151)	0.727
Angiopoetin-1 (ng/mL)	3113 (1956 – 5299)	3480 (2467 – 5460)	2870 (1868 – 5357)	3126 (1787 – 4473)	0.501
Angiopoetin-2 (ng/mL)	2668 (1558 – 4477)	2899 (2045 – 4914)	2825 (1482 – 4643)	2394 (1580 – 3648)	0.435
<b>Hematology and Blood</b>					



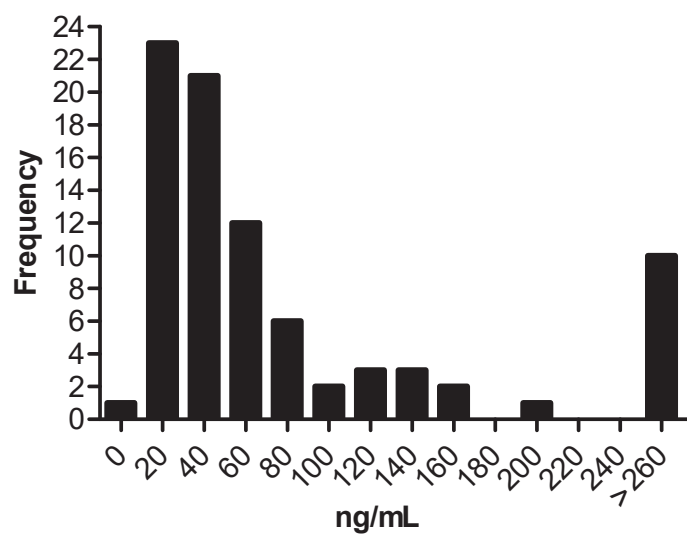
Sodium (mEq/L)	138 (136 – 140)	138 (135 – 140)	139 (136 – 140)	137 (136 – 141)	0.847
Albumin (g/dl)	39 (37 – 41)	40 (38 – 42)	38 (37 – 40)	40 (38 – 42)	0.05
Lymphocytes (x10 <sup>9</sup> /l)	1.3 (1.0 – 1.6)	1.4 (1.0 – 1.6)	1.4 (1.0 – 1.8)	1.3 (1.0 – 1.4)	0.533
Leukocytes (x10 <sup>9</sup> /l)	7.3 ± 2.5	7.9 ± 1.9	7.4 ± 2.5	6.6 ± 1.9	0.463
Neutrophils (x10 <sup>9</sup> /l)	4.6 (3.6 – 6.0)	5.4 (3.9 – 6.6)	4.0 (3.0 – 5.0)	4.8 (4.0 – 6.0)	0.079
Hematocrit (decimal fraction)	0.35 (0.32 – 0.37)	0.35 (0.33 – 0.37)	0.36 (0.32 – 0.38)	0.35 (0.32 – 0.37)	0.732
<b>Blood volume markers</b>					
BNP (pg/ml)	351 (176 – 759)	560 (208 – 923)	375 (227 – 740)	213 (119 – 390)	0.019
NT-proBNP (pg/ml)	3997 (1706 – 8605)	5774 (1970 – 10390)	4763 (2981 – 9867)	2494 (1176 – 5925)	0.066
Pro-ANP (pmol/l)	794 (557 – 1133)	834 (602 – 1249)	858 (645 – 1133)	597 (464 – 828)	0.024

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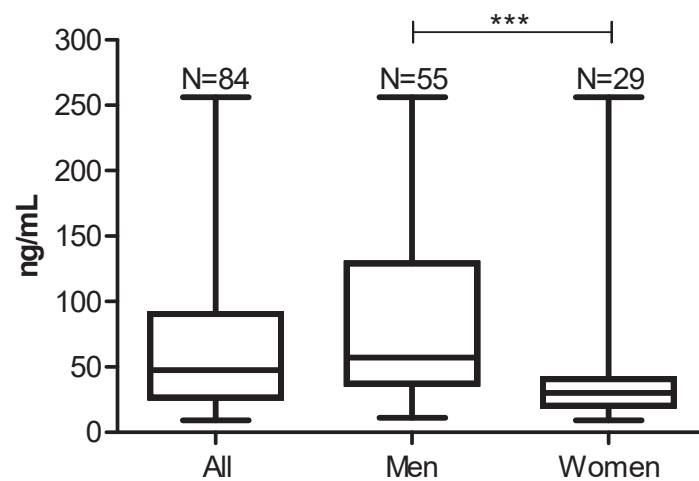
3 Normally distributed data are shown as means SD, skewed distributed data are shown as medians with interquartile ranges in parentheses, and  
4 categorical distributed variables are shown as numbers and percentages [n (%)]. Abbreviations: BMI: body mass index; BNP, brain natriuretic  
5 peptide; CRP: C-reactive protein; HD: hemodialysis; ICAM-1: Intercellular Adhesion Molecule 1; IL: interleukin; NT-proBNP: N-terminal pro b-  
6 type natriuretic peptide; N: number; pro-ANP: pro-atrial natriuretic peptide; PTX3: pentraxin-3; SGA: Subjective Global Assessment; TNF- $\alpha$ :  
7 tumor necrosis factor  $\alpha$ .

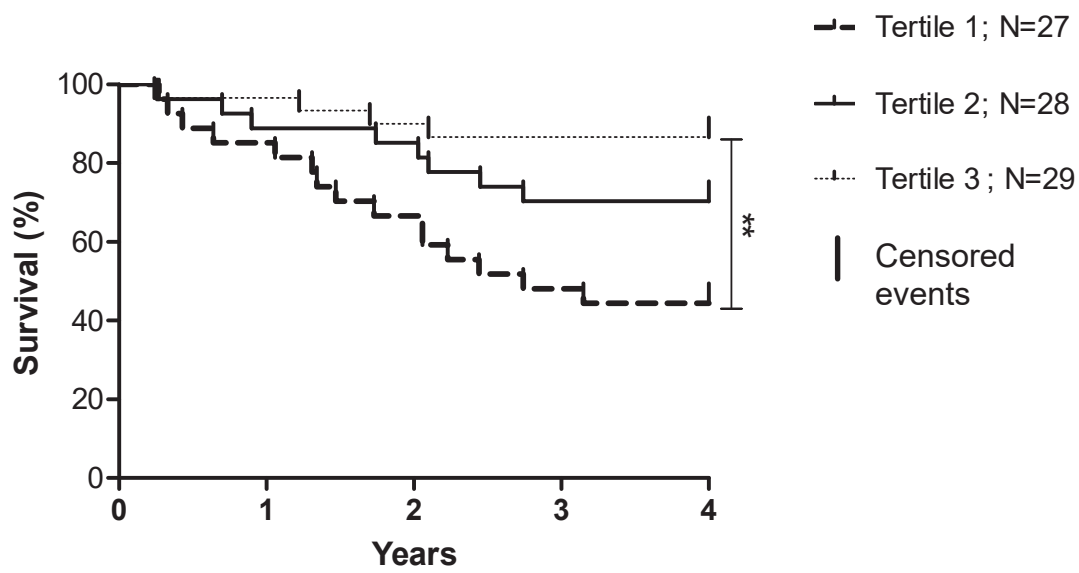
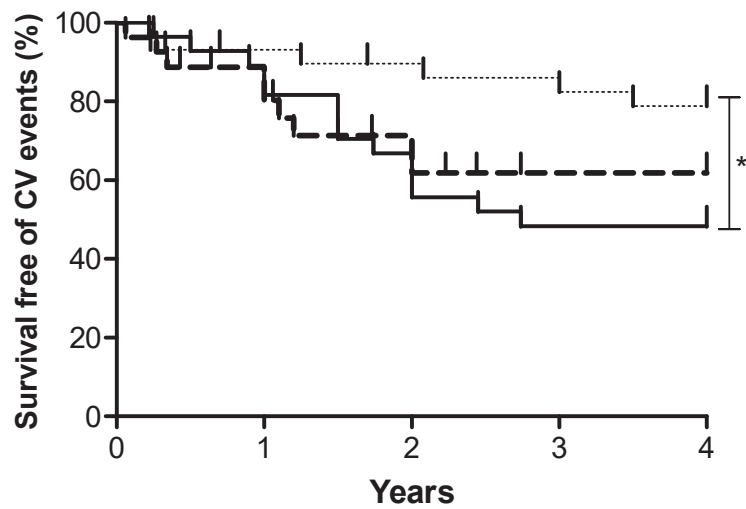
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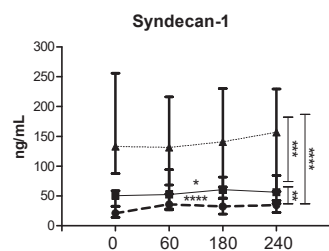
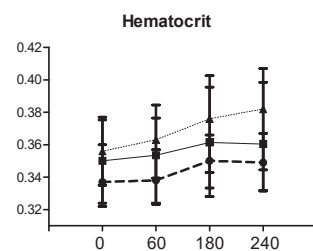
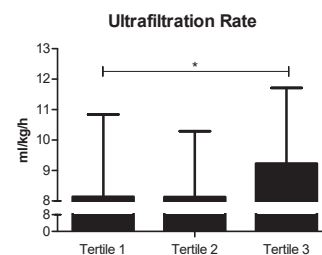
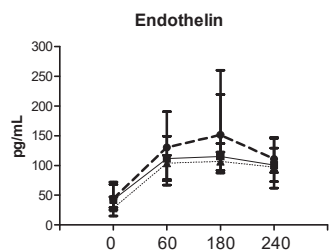
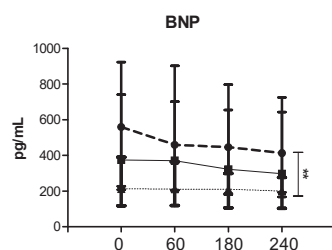
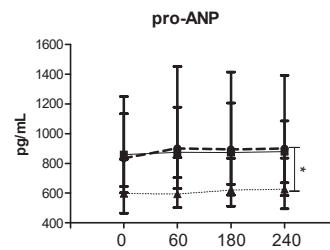
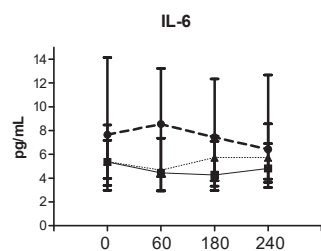
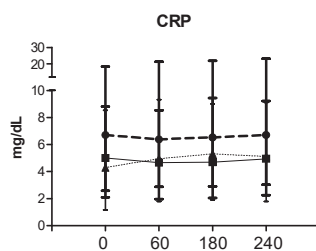
**a**



**b**



**a****b**

**a****b****c****d****e****f****g****h**

—●— Tertile 1; N=27  
 -■- Tertile 2; N=28  
 ...▲... Tertile 3; N=29

0, 60, 180, 240: Minutes after Hemodialysis